

# EXHIBIT F

**United States District Court**

**Northern District of California**

**Case No.: 3:18-cv-01586-JSC**

**IN RE PACIFIC FERTILITY CENTER LITIGATION**

**Expert Report of David Wininger, Ph.D.**

**November 6, 2020, Amended December 4, 2020**

**Exhibit F**

## TABLE OF CONTENTS

I. QUALIFICATIONS.....	1
II. ASSIGNMENT .....	2
III. BACKGROUND.....	3
A. What is Embryology? .....	3
B. The In Vitro Fertilization (IVF) Process.....	3
C. The Cryopreservation Process and Vitrification.....	6
D. Cryogenic Storage Containers .....	7
E. IVF Outcome Data .....	9
F. PFC’s Outcome Data .....	10
IV. OPINIONS .....	14
A. Tank 4 Failed to Perform as Safely as Ordinary Users Expect.....	14
B. The Eggs and Embryos Stored in Tank 4 Were Damaged .....	17
i. Tank 4 Embryos Fail to Successfully Thaw at Much Higher Rates .....	18
ii. Tank 4 Eggs Fail to Successfully Thaw at Much Higher Rates .....	19
iii. Tank 4 Transfers Are Less Likely to Lead to a Pregnancy .....	20
iv. The Cumulative Impact of Tank 4’s Lower Success Rates on Plaintiffs .....	21
C. Tank 4 Eggs or Embryos Were Not Damaged Before March 2018 .....	24
V. Materials Reviewed.....	28

## **I. QUALIFICATIONS**

1. For over 30 years, I have been an IVF Laboratory Director, an active embryologist, and an embryology consultant. I received my MS in Biotechnology in 1988 and my Ph.D. in Zoology in 1990 from the University of Tennessee. I have designed five IVF labs which involved working with architects, engineers, and air filtration experts. I am active in research and have published multiple scientific articles, book chapters, and abstracts. I have also presented at multiple scientific meetings. I am an active member of the American Society for Reproductive Medicine (ASRM), The American Association of Bioanalysis (ABB) and the College of American Pathologists (CAP), of which I have been an Inspector for over 20 years. I also served as Chair of the Validation Committee for the Society for Assisted Reproductive Technology (SART) for eight years.

2. After receiving my Ph.D. in 1990, I began directing an IVF lab in Charlotte, NC. I moved to Pennsylvania in 1993 and began directing the IVF lab at Abington Memorial Hospital. In 2008, I moved to Atlanta, GA and became the lab director at Reproductive Biology Associates, which is the largest fertility program in the Southeast. In 2003, I moved to North Carolina and became the lab director at Wake Forest University Baptist Medical Center. The first task I had at Wake Forest was to design their new IVF lab and oversee development of their first on-site genetic testing program for preimplantation genetic testing (PGT). In 2008, I was asked to design a new IVF lab in High Point, NC. During that design process I was offered the lab director position. The Medical Director and several nurses from Wake Forest also joined me at Premier Fertility Center, and we had a successful fertility program for ten years. I then became lab director of Atlantic Reproductive Medicine in Raleigh, NC. I have rewritten the embryology protocols and have retrained several of the embryologists there. I have also served as consultant

IVF lab director for multiple labs. I am currently a consultant lab director for labs in Pittsburgh, PA, Statesville, NC and Austin, TX.

3. All the labs with which I am associated are accredited by CAP and FDA, as well as holding state licensure. CAP and FDA inspections occur biannually. In preparation for CAP inspections, all checklists sent by CAP must be examined to ensure that all protocols, quality control, and quality control documents are in order. There must be evidence of active review of all IVF outcomes including ICSI, embryo biopsy, cryopreservation and pregnancy rates. There must be documentation of generator testing and monitoring of all cryopreserved embryo and oocyte storage tanks. The FDA examines all donor sperm and oocyte infectious disease testing documents and cryostorage.

## **II. ASSIGNMENT**

4. I was engaged by Plaintiffs' counsel in this action on behalf of IVF clients whose oocytes and embryos were in storage at PFC in a cryogenic storage tank labeled "Tank 4" as of March 4, 2018. On that date, laboratory staff at PFC discovered that Tank 4 had suffered a vacuum failure and loss of liquid nitrogen, resulting in the exposure of the tank's contents to a higher temperature than appropriate and to uncontrolled thaw conditions (the "Tank 4 incident").

5. I was retained to provide my professional opinion concerning the incident and its impact on Plaintiffs, and to provide background about the IVF process. Specifically, I was asked to opine as to (i) whether Tank 4 performed as safely as an ordinary user of cryogenic vessels would expect; (ii) whether Plaintiffs' eggs and embryos were damaged by the Tank 4 incident; and (iii) whether Plaintiffs' eggs and embryos were exposed to dangerous conditions prior to the Tank 4 incident.

6. I reserve the right to supplement my opinions and address the opinions of any expert engaged by the Defendant.

7. I have never testified as an expert consultant in litigation. My rates are \$450/hour for consulting and \$600/hour for testifying. My CV, including my publications, is attached as Exhibit A.

### **III. BACKGROUND**

#### **A. What is Embryology?**

8. Embryology is a subfield of developmental biology which is concerned with the formation, growth, and development of embryos. It deals with the prenatal stage of development beginning with the formation of gametes (sperm and eggs), then fertilization, formation of a zygote (a fertilized egg), and development of an embryo and fetus, through to the birth of a new individual.

#### **B. The In Vitro Fertilization (IVF) Process**

9. IVF is a method of Assisted Reproductive Technology (ART) which involves the fertilization of a woman's eggs outside of her body in a laboratory. The fertilized eggs, or embryos, are cultured in plastic dishes containing solution that mimics the environment in the fallopian tubes. The embryos are normally grown for 5-6 days in the laboratory at which time one may be transferred to the patient's uterus. This process is called an embryo transfer (ET). The embryos might be cryopreserved, or vitrified, for a future frozen embryo transfer (FET). The majority of clinics opt for cryopreservation of all embryos due to higher pregnancy rates when FETs are performed.

10. The first step of the IVF process is to produce multiple eggs in a patient's ovaries with the use of reproductive hormones, or fertility drugs. Normally a woman produces one egg in

a normal cycle but with ovarian stimulation using hormones, the number of eggs can be from 2-40 or more, based on the patient's age and the dose of hormones. During this process, the MD, who has training in Reproductive Endocrinology, monitors the growth of the follicles in the ovaries which contain the eggs. Monitoring involves blood hormone measurements (estrogen and progesterone) and ultrasounds. When the ovarian follicles reach 18-20 mm in diameter and the hormone measurements are appropriate, a final injection of a hormone called HCG is given. At this point, the eggs will be ready to be removed from the ovaries in 34-36 hours.

11. Prior to the removal of the eggs, or egg retrieval, an anesthesiologist sedates the patient in preparation for the procedure. The eggs are removed from the patient's ovaries by a Reproductive Endocrinologist with a needle which is attached to suction. All the follicles that contain the eggs are drained into sterile plastic tubes and are handed through a pass-through window into the IVF laboratory. There, an embryologist pours the fluid into large plastic dishes and locates the eggs. This process continues until all ovarian follicles have been drained.

12. While the embryologist is locating eggs, an andrologist is preparing the sperm sample for insemination, the addition of sperm to the eggs. The sperm sample can either be from the patient's spouse or a sperm donor. The sperm sample is prepared by "washing" it with a saline based solution. It is placed over a viscous solution and centrifuged to remove nonmotile sperm, white cells, and any other non-sperm cells that may be in the sample. This highly purified sample is put into an incubator at body temperature until it is needed in a few hours.

13. Following the egg retrieval, the oocytes are prepared for the insemination procedure, which will begin fertilization. The most common method of insemination is intracytoplasmic sperm injection (ICSI). The first step of this procedure is to determine how many of the eggs are mature, or at Metaphase II of Meiosis. Only eggs which are at this stage

have a chance of fertilizing. 3-4 hours after the egg retrieval, the mature eggs will be placed in drops of a specialized IVF solution, along with a small drop of the partner's or a donor's sperm sample and put on the stage of an inverted microscope. The sperm is picked up one at a time with a glass needle and injected into each oocyte. This process will continue until all eggs are injected. If ICSI is not ordered by the physician, then approximately 50,000 sperm will be added to each drop of 3-4 eggs. This process is known as conventional insemination and also occurs 3-4 hours after the egg retrieval.

14. The inseminated oocytes are moved to preequilibrated drops of IVF culture media drops in petri dishes that are covered with sterile oil. The oil holds the drops in place and also keeps them from evaporating because the drops are very small. The dishes are in temperature and humidity regulated incubators. The oocytes incubate for 16-18 hours and then are observed for signs of fertilization. Specifically, the oocytes are observed under a microscope for presence of two round structures called pronuclei. One structure represents the DNA of the oocyte and the other the DNA of the sperm. At this point, the fertilized eggs are known as zygotes.

15. The embryos remain in culture for five to six days, at which point they should reach the blastocyst stage. This is an advanced stage of embryo development and not all embryos make it to this point. Blastocysts are graded by their degree of expansion and also by the grade of two cell types that are found in blastocysts. The cell types are the inner cell mass (ICM), which will become the fetus, and the trophectoderm cells (TE) which will become the placenta, amnion, and chorion of the developing baby. There are three options for the use of the blastocysts.

16. First, one embryo can be transferred to the patient and the remainder vitrified. Second, all blastocysts can be vitrified and used later in a frozen embryo transfer (FET). The third option is to perform embryo biopsies on the blastocysts in order to determine the genetic

status of each one. Many IVF clinics perform PGT on all embryos of all of their patients.

Following biopsy, the biopsied cells are loaded into small tubes and placed in the freezer. They will later be sent to a genetics lab for analysis. The most common genetic test is PGT-A. This test basically ensures that there are two copies of every chromosome and either XX (female) or XY (male). Pregnancy rates with genetically normal embryos are much higher than without testing.

17. Two weeks after an embryo is transfer a blood test is performed for detection of the pregnancy hormone, HCG. HCG is secreted into the bloodstream by an embryo that has implanted in the uterus. If HCG is detected, the level is checked again in a week. If the HCG level has at least doubled, it will be followed two weeks later by an ultrasound for detection of a gestational sac and, later, by another ultrasound to detect a fetal heartbeat. Once a fetal heartbeat is detected, the patient will be referred to her OB/GYN for obstetrics care for the duration of the pregnancy.

### **C. The Cryopreservation Process and Vitrification**

18. Vitrification is the practice of cryopreserving an egg or embryo with extremely rapid cooling and has been preferred method for cryopreservation in the IVF laboratory for two decades. In the context of freezing eggs and embryos, vitrification cools the tissue so rapidly that that the water molecules within the tissue do not have time to form ice crystals. Instead, the cells of the tissue instantaneously transform into glass-like structures. This overcomes the primary difficulty with freezing any cell of the human body, which is that the fluid inside of the cells can form tissue-damaging ice crystals.

19. Vitrification uses an extremely quick cooling rate (approximately 15,000°C/min) for near-instantaneous transformation. Furthermore, vitrification suspends cryopreserved

samples in a lattice structure that does not have ice crystal formation as a side effect. Many studies have shown very minimal damage is caused by the vitrification process. Additionally, vitrification technology allows specimens to be stored indefinitely, with little or no negative impact from the length of time the sample is stored.

20. Both vitrification and the subsequent thaw of vitrified tissue require precise execution by a skilled embryologist. In particular, the tissue's exposure to liquid nitrogen and the cryoprotectants that are a necessary element of the process – but also toxic under certain conditions – must be carefully controlled. One such toxic cryoprotectant that is widely used to vitrify eggs and embryos is dimethyl sulfoxide, or DMSO.<sup>1</sup>

21. Both the rates of cooling and of thawing are critical for success with vitrification.<sup>2</sup> Preventing the formation of ice crystals during the thawing procedure is of paramount importance and the procedure must be completed quickly and under strict temperature control.<sup>3</sup> Ice crystal formation can occur during the warming process starting at -150°C through -132°C.<sup>4</sup> Properly performed vitrification and subsequent thaw avoids ice crystal formation, allowing complete survival and high viability rates of the vitrified tissue.

#### **D. Cryogenic Storage Containers**

22. IVF laboratories use cryogenic storage containers holding liquid nitrogen to maintain eggs and embryos in a vitrified state. There are several different shapes and sizes of

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<sup>1</sup> See Modulating the Structure and Properties of Cell Membranes: The Molecular Mechanism of Action of Dimethyl Sulfoxide, J Phys. Chem. B. 2007. <https://doi/10.1021/jp073113e>

<sup>2</sup> Factors affecting the outcome of human blastocyst vitrification  
Reprod Biol Endocrinol. 2009; 7: 99

<sup>3</sup> Id.

<sup>4</sup> The relevance of ice crystal formation for the cryopreservation of tissues and organs  
January 2010Cryobiology 60(3 Suppl):S36-44

cryogenic storage containers utilized by IVF labs, but they are all are thermally insulated with a vacuum jacket.

23. Liquid nitrogen maintains a temperature of  $-196^{\circ}\text{C}$ , and eggs and embryos are typically stored fully submerged within it. Below is an example of a cryogenic storage container used by PFC, shown from the side and from above:



24. The vacuum layer between a cryogenic container's inner and outer wall is what allows it to maintain the low temperatures that vitrified eggs and embryos require. That vacuum space impedes heat transfer from the room-temperature IVF laboratory to the interior of the tank, where the liquid nitrogen and cryopreserved tissue are stored at  $-196^{\circ}\text{C}$ .

25. Some of the cryogenic container's liquid nitrogen will warm up and evaporate over time, but only a relatively small portion, which can be replenished by adding more liquid nitrogen. The vacuum insulation used in cryogenic containers is so effective at impeding heat transfer, that it can take weeks for all the liquid nitrogen stored within a container to evaporate.

26. Cryogenic storage containers are a critical component of the IVF process as they regulate the temperature of the cryopreserved reproductive material and keep vitrified eggs and embryos much colder than -150°C, at which threshold damaging ice crystal formation can begin.

#### **E. IVF Outcome Data**

27. IVF clinics in the U.S. have been required to register themselves as IVF practitioners and report their treatment data to the CDC since 1992, either directly or through the Society for Assisted Reproductive Technology (SART).<sup>5</sup> SART requires clinics to provide hundreds of data fields that convey information about the clinics' patients, IVF cycles, and thaw attempts. This data must be reviewed by the reporting clinic's medical director, who verifies the authenticity of the information prior to submission.<sup>6</sup> SART then makes aggregate data available to the public through its website, where prospective IVF patients and other interested parties can review the IVF success rates achieved nationally and by individual fertility clinics.<sup>7</sup>

28. There are several discrete phases involved in a frozen embryo transfer, and success rates can be measured and reported for each of them. For instance, each time PFC attempted to thaw a frozen egg or embryo, it would record whether or not the tissue appeared to survive the thaw process when viewed under a microscope. The clinic's thaw success rate can be calculated from these numbers to report the percentage of frozen eggs or embryos that survive the thaw process.

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<sup>5</sup>The Fertility Clinic Success Rate and Certification Act,  
<https://www.cdc.gov/art/nass/policy.html>

<sup>6</sup> National ART Surveillance  
<https://www.cdc.gov/art/nass/index.html#accuracy>

<sup>7</sup> Understanding the SART Clinic Report  
<https://www.sart.org/patients/fyi-videos/understanding-the-sart-clinic-report/>

29. Following a successful thaw, an oocyte will typically undergo an insemination procedure, after which it will be incubated and any resulting embryo will be allowed to develop in culture for several days. Fertilized eggs that successfully develop into viable embryos are then either transferred to the patient's uterus, refrozen for later transfer, or discarded. Embryos do not need to undergo as complicated of a procedure upon thaw, but will likewise either be transferred, refrozen for a later transfer (for example if the embryos was thawed for PGT), or discarded (which while rare, could occur if prior to transfer or refreezing, the embryo no longer appeared viable under the microscope). PFC records the ultimate disposition of all eggs and embryos that it thaws, marking each as either transferred, refrozen, or discarded. The retention rate can be calculated to determine the percentage of eggs or embryos that both survive thaw and develop into viable embryos, providing another benchmark rate of success.

30. Two more benchmarks for IVF success that are typically tracked and reported are the percentage of embryos that result in a clinical pregnancy following transfer, and the percentage of clinical pregnancies that lead to at least one live birth. PFC tracks both of these outcomes for all frozen embryos transfers, which can be used to generate both an implantation success rate and a live birth success rate.

#### **F. PFC's Outcome Data**

31. To assess whether the eggs and embryos stored in Tank 4 at the time of the incident were damaged, I was asked to review the success rates for eggs and embryo thawed and/or transferred at PFC prior to the Tank 4 incident and after the incident. To facilitate this comparison, PFC produced several data sets.<sup>8</sup> One source of relevant data was a 360-column

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<sup>8</sup> MSO26787, MSO026942, MSO026859, MSO027014 (8/5/20), and MSO027014 (9/24/20). I understand that actual production occurred through Pacific MSO, LLC, which operates the PFC lab; for simplicity, I refer to PFC and Pacific MSO, LLC interchangeably in this report.

spreadsheet that PFC prepared from outcome data it downloaded from the SART portal.<sup>9</sup> This was certified data that PFC had provided to SART, but it had two significant limitations: it only covered the period between 2012-2018, and it only provided cycle-level data. PFC also provided comprehensive data from its internal eIVF database, which covered the period from January 1, 2012, through August 3, 2020; included both cycle-level data that covered all frozen embryo transfers and tissue-level data that covered all thaws associated with each cycle; and identified the PFC Tank where the tissue was stored prior to thaw.<sup>10</sup> PFC would later supplement the tissue-level data to identify any prior Tissue IDs associated with each thawed egg or embryo.<sup>11</sup> The prior Tissue IDs made it possible to identify not only whether a given egg or embryo was stored in Tank 4 at the time of thaw, but also whether an embryo had previously been stored in Tank 4 or was derived from an egg that had been stored in Tank 4. Finally, PFC produced a spreadsheet that one of its embryologists used to keep track of all thaws and transfers of any material stored in Tank 4 at the time of the Incident.<sup>12</sup>

32. PFC's data sets were synthesized into a single spreadsheet and provided to Nicholas Jewell, Ph.D., a professor of biostatistics, who calculated the success rates obtained by PFC patients before and after the Tank 4 incident and opined on the statistical significance of the respective success rates.<sup>13</sup> The data set provided to Professor Jewell included all columns from the eIVF data produced by PFC needed to determine a thaw success rate, a retention rate after thaw, an implantation success rate, and a live birth success rate. It also included potentially relevant data such as whether a donor egg was used or the patient's own eggs, the age of the egg

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<sup>9</sup> MSO26787

<sup>10</sup> MSO026942 (cycle-level data); MSO027014 (8/5/20) (tissue-level data)

<sup>11</sup> MSO027014 (9/24/20)

<sup>12</sup> MSO026859

<sup>13</sup> PFC Outcome Data 10-1-20.xlsx

at the time of retrieval, and gravidity. These factors may impact IVF success rates and were provided to Professor Jewell to permit him to test for any confounding effects in the data.<sup>14</sup> The data was compared against SART data prior to submittal to Dr. Jewell, and in the event of a conflict, the SART data was used unless it was evident from the surrounding data that the SART data was incorrect. The data was also compared against the Tank 4 spreadsheet to ensure that all tissue affected by the Tank 4 Incident had been properly identified as such.

33. The spreadsheet provided to Professor Jewell included four worksheets—one for blastocyst thaws, one for oocyte thaw, one for transfers, and one for pregnancies. The data PFC provided only identified the tissue at the time of its first freeze, so a manual review of the data was necessary to properly characterize frozen blastocysts that were previously frozen eggs. All blastocyst and oocyte thaws between January 1, 2020, and August 3, 2020, were included.

34. Transfer data covered the time period between January 1, 2020 to April 10, 2020. PFC stopped performing IVF transfers for several weeks after April 10, 2020, due to the COVID-19 pandemic, and the Treatment Outcome was incomplete for transfers performed in June, July, and August 2020, as it can take a few months for fertility clinics to obtain clinical pregnancy results from patients and enter the outcome in their internal databases. Pregnancy data covered the time period between January 1, 2020, and October 31, 2019, with more recent pregnancies excluded due to incomplete outcome data for women who may have still been pregnant on August 3, 2020 (when the data was compiled by PFC) or who had not yet reported the outcomes of their pregnancy to PFC.

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<sup>14</sup> An Analysis of the Effect of Age on Implantation Rates. *J Assist Reprod Genet* **17**, 303–306 (2000). <https://doi.org/10.1023/A:1009422725434>; Donor age is a major determinant of success of oocyte donation/recipient programme, *Human Reproduction*, Volume 27, Issue 1, January 2012, Pages 118–125, <https://doi.org/10.1093/humrep/der359>

35. Prof. Jewell also calculated and analyzed success rates for PFC patients with eggs or embryos in Tank 4 on December 30, 2013, and January 20, 2014, when I understand Chart contends Tank 4 may have run out of liquid nitrogen. A second spreadsheet was provided to Professor Jewell for this purpose that identified whether particular tissue was in the tank on those dates.<sup>15</sup> This spreadsheet also corrected the [REDACTED]

[REDACTED]  
and corrected the [REDACTED]  
[REDACTED]  
[REDACTED]

[REDACTED] These errors in PFC's data were corrected before Professor Jewell included any 2012-13 thaws or transfers in his analysis.

36. I have reviewed the various data sets produced by PFC as well as the spreadsheets used by Prof. Jewell to conduct his analysis. I believe the spreadsheets used by Prof. Jewell were appropriately prepared and that appropriate efforts were made to correctly address inconsistencies in PFC's data. I have also reviewed Prof. Jewell's analysis of PFC's outcome data, and believe that the potential confounding variables he considered in his analysis—namely, egg age, egg source, and gravidity—were the appropriate factors to consider when assessing whether differences between the types of eggs and embryos stored in Tank 4—rather than the Tank 4 Incident—could be responsible for the poor success rates that have been achieved to date using Tank 4 tissue.

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<sup>15</sup> PFC Outcome Data 10-19-20.xlsx

#### IV. OPINIONS

##### A. Tank 4 Failed to Perform as Safely as Ordinary Users Expect

37. I have worked with cryogenic storage containers for more than 30 years. I have worked with many types and manufacturers of cryogenic storage containers. It is my opinion that Tank 4 did not perform as safely as an ordinary user of cryogenic storage containers, like myself and the embryologists I work with every day, would expect it to perform when used or misused in an intended or reasonably foreseeable way.

38. In my experience, ordinary users of cryogenic storage tanks expect them to be capable of safely storing sensitive biological samples at cryogenic temperatures for a minimum of ten years. I agree with Chart's Cryobio Product Specialist, Ramón Gonzalez, in this regard.

Mr. Gonzalez states that Chart freezers [REDACTED]

[REDACTED]<sup>16</sup> As Mr. Gonzalez points out, [REDACTED]

[REDACTED]

[REDACTED]<sup>17</sup> Users do not expect the vacuum layer to degrade to the point the tank needs to be replaced or re-vacuumed prior to ten years.

39. Users of cryogenic tanks, like myself, also do not expect them to suffer a sudden and total loss of vacuum insulation (as opposed to a gradual degradation of the vacuum layer). Nor do they expect that the tank could consume more than 14 inches of liquid nitrogen in less than 24 hours—which is what happened to Tank 4. As one a regulatory representative for Chart

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<sup>16</sup> CHART050770

<sup>17</sup> Id.

put it, [REDACTED]

[REDACTED]<sup>18</sup> One of Chart's Biomedical Quality Engineers similarly affirmed that Chart's freezers "can maintain LN2 (liquid nitrogen) and temperature for quite a while, for weeks on end."<sup>19</sup> And Chart's medical risk management team likewise emphasized that "Chart vacuum-insulated vessels provide hold times of at least 7 DAYS."<sup>20</sup> The risk management team assigned the possibility of total vacuum loss like that suffered by Tank 4 to the lowest possible risk level: "So unlikely, occurrence not expected."<sup>21</sup> Based on my 30 years of experience working with cryogenic vessels, I agree with those assessments. No reasonable user of cryogenic containers expects the tank to suffer a total vacuum loss and suddenly lose its ability to maintain sensitive biological samples at cryogenic temperatures.

40. The total loss of a cryogenic tank's vacuum insulation is extremely hazardous for the biological materials stored inside, which are at a severe risk of cellular damage or death when exposed to warmer temperatures. The threat is especially severe because unlike gradual vacuum failures, total vacuum failures are not preceded by warning signs that could allow preventative action to be taken. A tank that has been in use for more than ten years and is losing vacuum due to gradual degradation will display observable physical symptoms. [REDACTED]

[REDACTED]<sup>22</sup> None of the many

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<sup>18</sup> CHART055224 at 55230

<sup>19</sup> 1/14/20 Junnier Dep. 72:14-73:07

<sup>20</sup> CHART001432, DFMECA, N6

<sup>21</sup> CHART001432, DFMECA, P47; Risk Estimation, K6

<sup>22</sup> See 1/23/20 Brooks Dep. at 139

embryologists who interacted with Tank 4 in the days, weeks, or months prior to its failure observed any such symptoms, according to their deposition testimony.

41. Also indicative of Tank 4's failure to perform as safely as an ordinary user like myself would expect was its physical appearance after it failed. The significant distortion of the inner tank wall, as shown in the photograph below, is highly unusual and not something that a typical user of cryogenic containers has likely ever encountered. I have worked with cryogenic containers for 30 years and have never seen or heard of a vessel deforming in the way that Tank 4 deformed. The PFC embryologists who saw Tank 4 after it failed affirmed that they too had never seen a tank deform in that way and did not even know that cryogenic tanks could fail in that manner.<sup>23</sup>

42. For all of these reasons, I am of the opinion that Tank 4 did not perform as safely as an ordinary user of cryogenic storage containers would have expected.

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<sup>23</sup> See, e.g., 8/31/20 Crimele Dep. at 131-32; 10/9/19 Conaghan Dep. at 143; 8/27/20 Fischer Dep. at 173-74; 8/28/20 Buchanan Dep. at 47



**B. The Eggs and Embryos Stored in Tank 4 Were Damaged**

43. As an embryologist, it would be extremely concerning to me if I knew that a patient's eggs or embryos were potentially exposed to temperatures warmer than  $-150^{\circ}\text{C}$ . As previously explained, when vitrified eggs and embryos are exposed to temperatures between  $-150^{\circ}\text{C}$  and  $-132^{\circ}\text{C}$ , ice crystals are likely to form within the eggs and embryos—leading to significant cellular damage or death.

44. I have reviewed the IVF success rates achieved by PFC patients using eggs and embryos that were stored in Tank 4 at the time of the incident on March 4, 2018.<sup>24</sup> These rates are [REDACTED] than the success rates typically achieved by PFC and confirm that the Tank 4 incident did indeed cause considerable damage to the eggs and embryos stored in Tank 4 at the time—including Plaintiffs' eggs and embryos.

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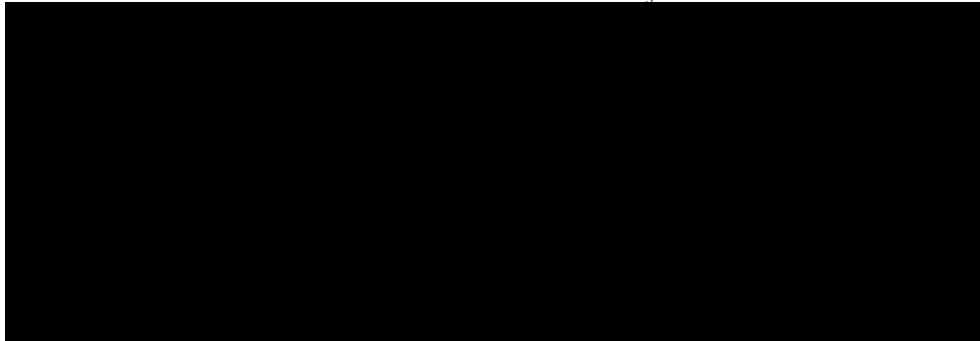
<sup>24</sup>11/6/20 Expert Report of Nicholas P. Jewell, Ph.D.

**i. Tank 4 Embryos Fail to Successfully Thaw at [REDACTED]**

45. The tables below compare PFC's Pre-Incident success rates with the clinic's Post-Incident success rates. The Pre-Incident rates were calculated from outcome data for PFC patients who started an IVF cycle in 2017 using their own eggs, while the Post-Incident rates are calculated from outcome data for PFC patients who had eggs or embryos stored in Tank 4 on March 4, 2018.

46. The first table summarizes the thaw success rates for frozen embryos from the Pre-Incident and Post-Incident populations:

**Thaw Success Rates - Embryos<sup>25</sup>**



47. The % Survived Thaw row shows the percentage of embryos that PFC recorded as surviving the thaw process, while the % Viable for Transfer row shows the percentage of embryos that were deemed suitable for transfer and were either transferred at that time or refrozen for later use; the remaining embryos were marked by PFC as discarded. Under ordinary circumstances, nearly all frozen embryos should survive the thaw process and be suitable for implantation. But after the Tank 4 incident, [REDACTED] of the embryos were found to have survived the thaw process and [REDACTED] were suitable for transfer [REDACTED]

[REDACTED]  
[REDACTED] When given

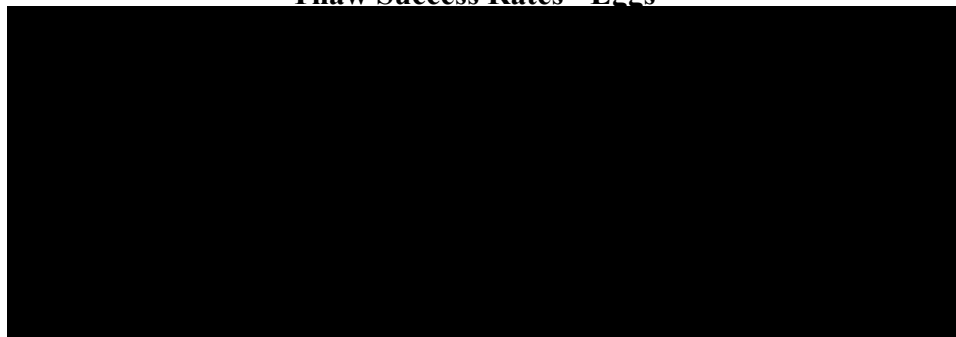
<sup>25</sup> 11/6/20 Expert Report of Nicholas P. Jewell, Ph.D., Tables 1-2

the chance to equilibrate, the cell structure collapsed and it became apparent that the embryo was dead. In fact, as explained below, PFC likely transferred dead embryos on several occasions but did not realize it at the time because the tissue was not given enough time to equilibrate. It is therefore likely that the true survival rate for Tank 4 tissue is [REDACTED]. The fact that the survival rate and viability rates of Post-Incident thaws are [REDACTED] than the typical and expected rates is an unmistakable indication that the Tank 4 incident severely damaged the tissue stored in Tank 4 at the time—ultimately rendering the [REDACTED] of the tissue non-viable for use in an IVF transfer.

**ii. Tank 4 Eggs Fail to Successfully Thaw at [REDACTED]**

48. The next table summarizes the thaw success rates for frozen eggs from the Pre-Incident and Post-Incident populations:

**Thaw Success Rates - Eggs<sup>26</sup>**



49. As explained above, oocytes are more fragile than embryos and pose a greater challenge to successfully cryopreserve. So whereas PFC's historical success rate at thawing embryos was [REDACTED] its success rate when thawing eggs, [REDACTED]. [REDACTED] Eggs exposed to the Tank 4 incident, however, hav [REDACTED] rived the thaw procedure [REDACTED] of the time—almo [REDACTED] than expected. This is another strong indication that Tank 4 tissue was indeed damaged by the incident.

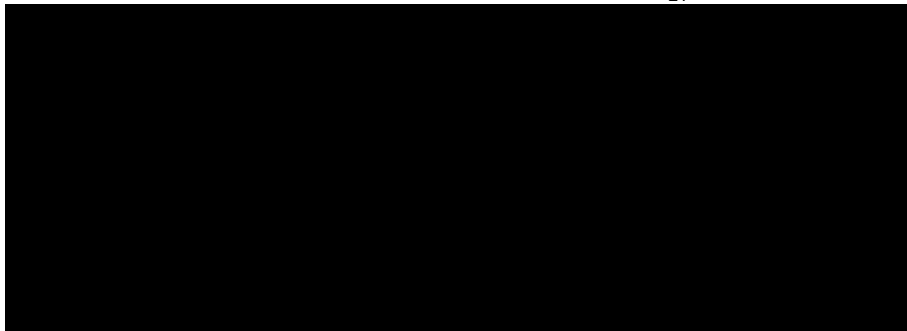
<sup>26</sup> 11/6/20 Expert Report of Nicholas P. Jewell, Ph.D., Tables 5-6

50. The % Viable for Transfer row reflects the percentage of oocytes that survived the thaw procedure, were successfully fertilized, and developed into an embryo suitable for transfer. Historically, PFC patients could expect to get roughly [REDACTED] viable embryo for every [REDACTED] frozen eggs. After the Tank 4 incident, however, the viability rate [REDACTED] so that now it would take an average of [REDACTED] frozen eggs to create [REDACTED] viable embryo.

**iii. Tank 4 Transfers Are [REDACTED] to Lead to a Pregnancy**

When tissue exposed to the Tank 4 incident has survived the thaw process and developed into an embryo deemed suitable for transfer, the ensuing transfer has led to some pregnancies and live births—but at a significantly lower rate than Pre-Incident transfers.

27



51. The pregnancies and live births achieved from Tank 4 embryo transfers are [REDACTED] of the Pre-Incident success rates. This is yet another indication that the Tank 4 tissue was damaged by prolonged exposure to high temperatures on March 4, 2018. It is likely that a significant number of the Tank 4 transfer attempts used embryos that appeared potentially viable but were actually dead. Tank 4 embryos were often transferred shortly after thaw, but it can take several hours for cell death to become apparent under the microscope.

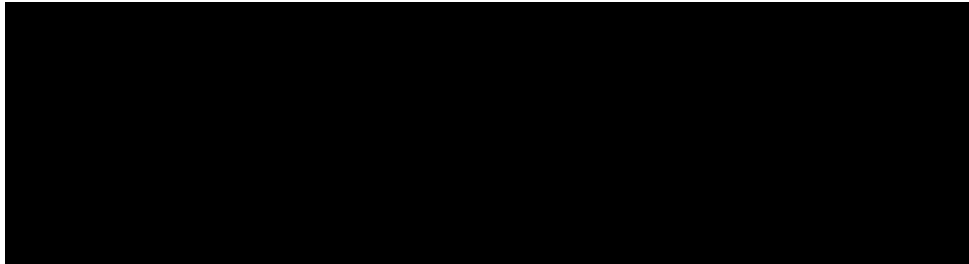
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<sup>27</sup> 11/6/20 Expert Report of Nicholas P. Jewell, Ph.D., Tables 7-8

iv. **The Cumulative Impact of Tank 4's [REDACTED] Success Rates on Plaintiffs**

52. The [REDACTED] success rates obtained from Post-Incident Tank 4 eggs and embryos are cumulative, and the end result for affected PFC patients is a [REDACTED] chance of obtaining a live birth. The table below shows the comparative likelihood that a frozen egg or frozen embryo would lead to a live birth before and after the incident.

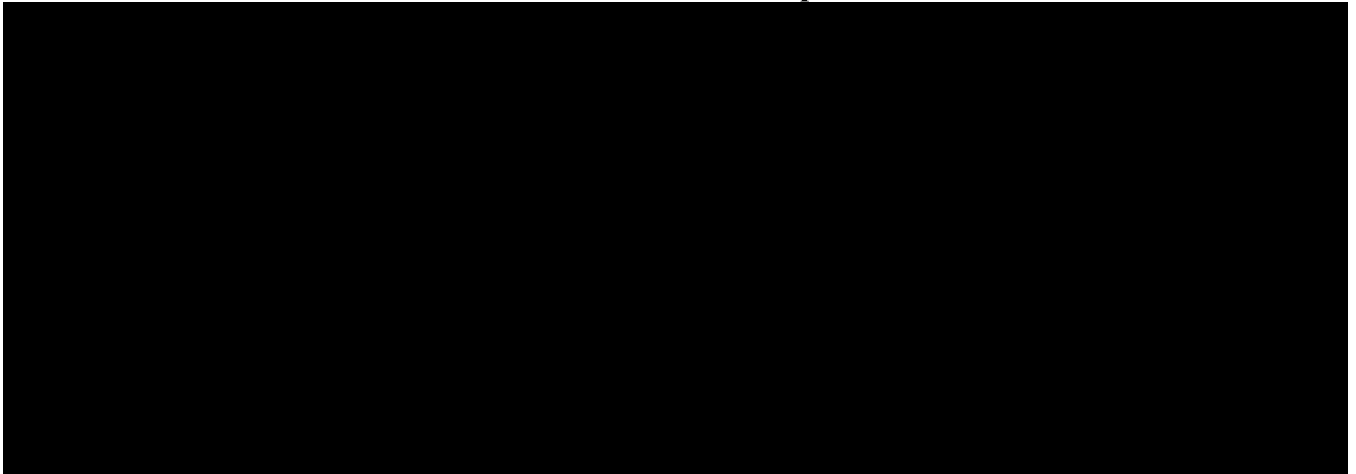
**Overall Success Rates<sup>28</sup>**



53. For both eggs and embryos, the chance of a live birth has been [REDACTED] by the Tank 4 incident. The chance that a frozen egg will lead to a live birth is about [REDACTED] than it was prior to the incident. And the chance that a frozen embryo will lead to a live birth is about [REDACTED]

54. The impact for Plaintiffs can be similarly quantified using the number of eggs or embryos each Plaintiff had stored in Tank 4. The table below shows (i) the average results I would have expected Plaintiffs to achieve Pre-Incident based on the success rates achieved by PFC patients with similar egg ages who attempted frozen embryos transfer at PFC between 2012-20 (excluding tissue affected by the Tank 4 Incident); and (ii) the average results I would expect Plaintiffs to achieve Post-Incident based on the success rates achieved by those PFC patients who attempted to use eggs or embryos stored in Tank 4 at the time of the Incident. The Post-Incident results are calculated using overall success rates achieved using Tank 4 tissue, as Prof. Jewell did not find that the [REDACTED]

<sup>28</sup> 11/6/20 Expert Report of Nicholas P. Jewell, Ph.D., ¶¶ 76-78.

**Effect of Tank 4 Incident on Plaintiffs' Expected Success Rates<sup>29</sup>**

55. Plaintiff E.F., for example, had 9 frozen eggs that were retrieved when she was 34 years old. Prior to the incident, she could have expected an average of [REDACTED] live births from those frozen eggs and a [REDACTED] chance of obtaining at least one live birth. After the incident, however, Plaintiff E.F. can [REDACTED] expect an average of [REDACTED] live births from her eggs and she has [REDACTED] a [REDACTED] chance of obtaining at least one live birth.

56. Were she to choose to use these damaged eggs, E.F. would be required to undergo the same physically, mentally, and emotionally exhausting process as if she were transferring healthy tissue. It is not advisable to thaw the tissue beforehand to see if it survives, and only then undergo the protracted series of hormones, injections, blood tests, and vaginal ultrasounds necessary for a frozen embryo transfer. The reason is that each time an egg or embryo is frozen it suffers some amount of cellular damage. That damage is typically insignificant on the first freeze, but each additional freeze adds risk—particularly when it is known that the tissue has already been affected by the Tank 4 incident. [REDACTED]

[REDACTED]

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<sup>29</sup> 11/6/20 Expert Report of Nicholas P. Jewell, Ph.D., Tables 2, 6, 8, 23, 25-26

[REDACTED]<sup>30</sup>

As an embryologist, I too would strongly recommend against thawing and refreezing Tank 4 tissue.

57. Plaintiff E.F. also would be required to accept the added risk that a baby born using damaged Tank 4 tissue will suffer adverse consequences after birth. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]<sup>31</sup> As PFC's President, Carl M. Herbert, M.D., explained, there is no information in the scientific or medical literature about what clinical or developmental consequences may arise as a result of using Tank 4 tissue: "Can you imagine the experiment where you thaw a human embryo uncontrollably and then try to make a baby out of it? I don't think so."<sup>32</sup>

58. For example, as discussed above, some of the cryoprotectants used in the freezing process have been shown to be toxic to animals.<sup>33</sup> Under normal IVF conditions, eggs and embryos are not meaningfully exposed to these cryoprotectants—they are only very briefly and precisely exposed during the freezing process and are not at risk while in the vitreous state. No testing has been done on whether thawed eggs or embryos exposed to these cryoprotectants for a prolonged period under uncontrolled conditions suffer harm.

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<sup>30</sup> MSO011539, MSO011573

<sup>31</sup> MSO\_PWCK\_000069

<sup>32</sup> 10/01/2019 Herbert Dep, 240:8-10

<sup>33</sup> "Dimethyl sulfoxide (DMSO) produces widespread apoptosis in the developing central nervous system". Neurobiology of Disease. 34 (1): 1–10. doi:10.1016/j.nbd.2008.11.006. PMC 2682536. PMID 19100327

59. Of the babies that have been born using Tank 4 tissue, [REDACTED] were born with low birthweights—about [REDACTED] the normal rate.<sup>34</sup> Low birthweights are associated with increased risk for a variety of health problems throughout one's lifetime.<sup>35</sup>

60. The uncontrolled and largely unprecedented exposure of Tank 4 tissue to high temperatures and cryoprotectants, the [REDACTED] success rates achieved from Tank 4 tissue throughout the IVF process, and the [REDACTED] rate of low birthweights that have resulted from Tank 4 pregnancies to date, all raise serious concerns about the long-term risks associated with the transfer of embryos affected by the Tank 4 incident. The decision of whether to use Tank 4 tissue is ultimately up to the patient, but as an embryologist, I would have serious reservations about using Tank 4 tissue to attempt a frozen embryo transfer.

### **C. Tank 4 Eggs or Embryos Were Not Damaged Before March 2018**

61. I understand that Chart contends that the tissue in Tank 4 may have been damaged prior to the March 2018 incident. In particular, Chart has pointed to two days—December 30, 2013, and January 20, 2014—when it says Tank 4's electronic controller recorded liquid nitrogen levels of 0 inches, and one day—February 26, 2018—when the controller was disabled but Chart's estimates indicate the liquid nitrogen dropped to 0.90 inches.<sup>36</sup>

62. I have reviewed the testimony from the embryologists working in or around Tank 4 on those dates, the log data from PFC's electronic controller in 2013 and 2014, the manual liquid nitrogen level logs kept by PFC in February and March 2008, and the success rates calculated by Dr. Jewell for tissue that was stored in Tank 4 on the dates in question and used prior to the March 2018 Tank 4 incident. I find no evidence that the eggs or embryos stored in

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<sup>34</sup> <http://dx.doi.org/10.15585/mmwr.ss6804a1> MMWR Surveill Summ 2019;68(No. SS-4):1–23

<sup>35</sup> Id.

<sup>36</sup> 01/10/20 Expert Report of K. Gustafson, ¶¶ 20-24, 33

Tank 4 were damaged by exposure to high temperature or other adverse environmental conditions prior to March 4, 2018.

63. The embryologists who were working in or around Tank 4 on December 30, 2013, or January 20, 2014, have testified that Tank 4 did not run out of liquid nitrogen and they would have been aware if it had. Having designed and directed multiple IVF labs, I can attest that if one of the cryogenic storage containers ran out liquid nitrogen, that would be a very serious event and one that everyone working in the IVF lab that day would be likely to know about and remember—much as occurred when Tank 4 did run out of liquid nitrogen on March 4, 2018.

64. In addition, as Chart has acknowledged, a log level reading of 0” does not mean that there is no liquid nitrogen left in the tank. The electronic controller could be incorrect. It also could mean only that there was less than 1.3 inches of liquid nitrogen in the tank. Tank 4’s electronic controller had a level offset of 1.3 inches in 2013 and 2014, which means that it will display and record a 0” reading when the liquid nitrogen level falls to 1.3 inches or below.<sup>37</sup> Eggs and embryos are typically stored in the very bottom of cryogenic storage straws, which are in turn stored in the very bottom of the cryogenic container. In the case of Tank 4, Dr. Conaghan has affirmed that the affected tissue was stored in a single layer on the bottom few millimeters of the tank, and embryologist Erin Fischer testified similarly.<sup>38</sup> Even if the recorded liquid nitrogen level dropped below an inch, the samples would still be submerged in liquid nitrogen and not exposed to dangerously high temperatures. [REDACTED]

[REDACTED]

<sup>37</sup> *Id.* ¶ 24; 02/06/20 Gustafson Dep., 267:19-268:21

<sup>38</sup> 10/09/19 Conaghan Dep., 121:8-18; 08/27/20 Fischer Dep., 99:7-14.

<sup>39</sup> In fact, the temperature readings recorded by Tank 4's electronic controller in [REDACTED]

<sup>40</sup>

65. With respect to the February 26, 2018 date, Chart's estimates of the liquid nitrogen level are based on the assumptions for daily liquid nitrogen consumption, liquid nitrogen fill rates, and the volume of samples in the tank that can vary from day to day and tank to tank, and therefore are unlikely to be accurate assumptions.<sup>41</sup> During this time period, Tank 4's electronic controller was [REDACTED]

<sup>42</sup>

66. To track Tank 4's liquid nitrogen levels while the electronic controller was malfunctioning, [REDACTED]

<sup>43</sup> I have

reviewed those logs and find that they evidence much higher levels of liquid nitrogen than those estimated by Chart.

67. If the eggs and embryos stored in Tank 4 had been exposed to dangerously high conditions as Chart contends, I would expect that the success rates achieved with tissue stored in Tank 4 on December 30, 2013, or January 20, 2014, and used prior to March 4, 2018, would be significantly lower than the success rates achieved by PFC using other tissue. In fact, Dr. Jewell found [REDACTED] between those success rates.<sup>44</sup>

<sup>39</sup> See CHART016303 at 16342, 16397

<sup>40</sup> CHART070093

<sup>41</sup> 01/10/20 Expert Report of K. Gustafson, ¶¶ 27-28

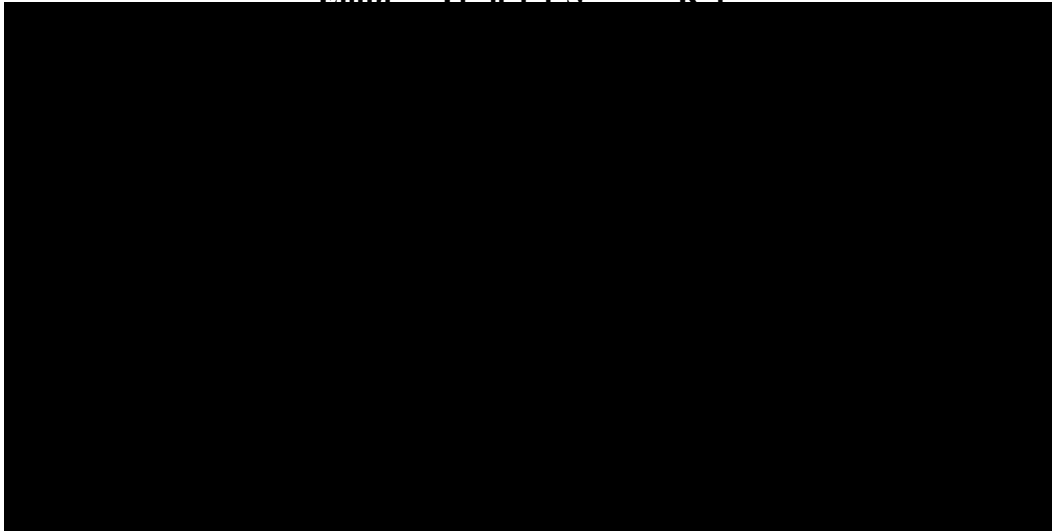
<sup>42</sup> MSO001982 at 1986

<sup>43</sup> Id.

<sup>44</sup> 11/6/20 Expert Report of Nicholas P. Jewell, Ph.D., ¶ 43.

68. The table below shows the success rates achieved with “exposed” tissue that was in Tank 4 on December 30, 2013 or January 20, 2014 (but not on March 4, 2018), alongside the success rates achieved by PFC for all patients in 2013. If anything, the “exposed” tissue performed better than the 2013 baseline.

Embryo Transfer Success Rate<sup>45</sup>



69. As an embryologist, these numbers are conclusive evidence that the eggs and embryos stored in Tank 4 on December 30, 2013, or January 20, 2014, were not exposed to dangerously high temperatures or otherwise affected by adverse environmental conditions.

70. [REDACTED] embryos were thawed between the third date that Chart claims Tank 4’s liquid nitrogen levels dropped near zero, on February 26, 2018, and the March 4, 2018 Incident. But it is notable that [REDACTED] embryos—[REDACTED] made from patient eggs and [REDACTED] made from donor eggs—survived the thaw process and were deemed suitable for transfer.<sup>47</sup> If tissue in Tank 4 was damaged on February 26 instead of March 4, it is exceedingly unlikely that [REDACTED] embryos would

<sup>45</sup> 11/6/20 Expert Report of Nicholas P. Jewell, Ph.D., Tables 11-12, 17-20

<sup>46</sup> All numbers are for patients who supplied their own egg with the exception of egg thaws, which are reported for patients who used donor eggs. Only 2 cycles of patient eggs were thawed in 2013, making a comparison impractical.

<sup>47</sup> Cycles 32512, 33009, 33236, 33276, 33346, 33380, 33443, 33543

have remained viable for transfer. Prof. Jewell calculated that the damaged embryos made from patient eggs have [REDACTED] chance of remaining viable upon thaw and damaged embryos made from donor eggs have [REDACTED] chance. The chance that [REDACTED] embryos would be viable if they were in fact damaged is therefore approximately [REDACTED]<sup>48</sup> Based on the success achieved thawing these [REDACTED] embryos, along with the Reflections logs indicating that liquid nitrogen was refilled and manually measured on a daily basis, it is apparent to me as an embryologist that Tank 4 tissue was damaged on March 4, 2018, and not on February 26, 2018, or any other earlier date.

## V. Materials Reviewed

§ Second Amended Consolidated Class Action Complaint

§ A.A. v. Chart Complaint

§ SART data published at [www.SART.org](http://www.SART.org)

§ CDC data published at [www.CDC/ART.org](http://www.CDC/ART.org)

§ August 27, 2018 Response by ASRM to Plaintiffs' Subpoena for Production of Documents, including responsive documents

§ 09/10/19 Deposition of Pacific MSO and Alden Romney

§ 10/01/19 Deposition of Dr. Carl Herbert

§ 10/08/19 Deposition of Caitrin Best

§ 10/09/19 Deposition of Pacific MSO and Joseph Conaghan

§ 12/04/19 and 02/11/20 Deposition of PFC and Eldon Schriock

§ 01/14/20 Deposition of Justin Junnier

§ 01/23/20 Deposition of Jeff Brooks

§ 2/06/20 Deposition of Keith Gustafson

§ 08/27/20 Deposition of Erin Fischer

§ 08/28/20 Deposition of Jennifer Andres

§ 08/28/20 Deposition of Kathrin Buchanan

§ 08/31/20 Deposition of Gina Cirimele

§ 09/02/20 Deposition of Jinnuo Han

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<sup>48</sup>  $0.44 \times 0.44 \times 0.44 \times 0.44 \times 0.38 \times 0.38 \times 0.38 \times 0.38 = 0.0008$  or 0.08%

§ 09/02/20 Deposition of Jean Popwell  
§ 09/09/20 Deposition of Pacific MSO and Joseph Conaghan  
§ 09/29/20 Deposition of Hana Lamb  
§ 10/17/20 Deposition of Anya Sokolova  
§ CHART000088 (tank drawing)  
§ CHART001432  
§ CHART0016303  
§ CHART050770  
§ CHART055224  
§ CHART070093 (10/18/2019-134119 Maximum Event Log)  
§ MSO000065-79 (Photos of Tank 4)  
§ MSO00310-363 (Reflections Data)  
§ MSO01982-2220 (March 23, 2018 Letter from Joseph Conaghan to Dr. Desiree Carlson with exhibits)  
§ MSO011539  
§ MSO011573  
§ MSO025536-25596  
§ MSO025597-25600  
§ MSO026787  
§ MSO026859  
§ MSO26942  
§ MSO026943  
§ MSO027014 [08/05/20]  
§ MSO027014 [09/24/20]  
§ PFC Outcome Data – 10-1-2  
§ PFC Outcome Data – 10-19-20  
§ MSO\_PWCK\_000069  
§ 01/10/20 Expert Report of Keith Gustafson  
§ 11/06/20 Expert Report of Nicholas P. Jewell, Ph.D.

Dated 12/4/2020

Signed 